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TITLE: Disaster Relief and Emergency Medical Services (DREAMS):
        Texas A&M Digital EMS and the Detection and Remediation
        of Chemical Threat Agents

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The Disaster Relief and Emergency Medical Services (DREAMS™) project is a consortium of scientists, medical professionals, and engineers from The Texas A&M University System and the University of Texas Health Science Center at Houston. The goal of DREAMS is to improve the diagnosis and treatment of critically ill or injured soldiers in the field by expediting their access to medical experts at trauma centers or field hospitals.

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INTRODUCTION

The Disaster Relief and Emergency Medical Services (DREAMS™) project is a well established consortium of molecular research scientists, emergency medical professionals, mechanical engineers, and computer l scientists from The Texas A&M University System in College Station and the University of Texas Health Science Center at Houston. The primary goals of the DREAMS project are to improve the real-time diagnosis and treatment of critically ill or injured patients by expediting the access of "first responders" to medical experts at trauma centers or field hospitals and providing accurate detection of the presence of chemical and biological threat agents. Texas A&M University System research scientists and engineers are working on two components of the DREAMS program:

1. Texas A&M University System (TAMUS) Digital EMS, and
2. Detection and Remediation of Chemical Threat Agents.

Texas A&M University System Digital EMS
Texas A&M Digital EMS is the DREAMS component that allows trauma specialists to treat patients more quickly by providing the "virtual" presence of a physician on the battlefield or at the emergency scene. Digital EMS focuses on integrating existing and "state-of-the-art" developing technologies into the DREAMS Interact demonstration ambulances. These include multiple leading-edge telecommunications technologies, including video processing, wireless communications, and innovative uses of "Commercial Off The Shelf" (COTS) digital technology. The Digital EMS ambulances connect emergency medical personnel on the scene with trauma specialists in distant hospitals. This allows physicians to monitor patients using real-time video and vital signs data from a suite of advanced digital medical monitoring equipment normally only available in a clinic or hospital.

Detection and Remediation of Chemical Threat Agents
The component of DREAMS dedicated to the detection and remediation of chemical and biological threat agents that is critical to the welfare of the soldier in the field and citizens at home. One of the newly emerging concerns regarding military and civilian disaster response scenarios is the involvement of chemical or biological weapons of mass destruction, which might range from noxious chemical fumigants to neurotoxic, chemical warfare agents, or from infective biological spores and viruses or their biologically-active toxins. Texas A&M scientists are developing genetically engineered enzymes that recognize and decontaminate a host of chemical and biological threat agents, and methods to integrate these new materials into detection and decontamination systems.

BODY

The Texas A&M University System Digital EMS

Task 1: Development of Site Specific Test Plans
The Texas A&M University System (TAMUS) continued to meet periodically with the Liberty County EMS to review operational and testing protocols for deployment. The test protocols and operational protocols with support documents were updated to reflect developments during the year. Coordination with Liberty County EMS for the first ambulance fielding was updated.

Meetings continued to be held with Liberty County Hospital to establish requirements for their participation as a rural hospital deployment site. These discussions were greatly protracted by requirements to satisfy their owners and insurers. Final agreements were not completed in project year 2004 due to issues with hospital insurance and proposed establishment of a county hospital district. Discussions will be resumed after the completion of the County hospital district referendum in February 2005.

In October, four Liberty County EMS lead paramedics were trained on the use of the paramedic station on the Digital EMS ambulance. Several scenarios were played out to fully evaluate the usefulness and benefits of the DREAMS technology. A detailed training manual depicting the various interface screens and their intent was provided to each paramedic as well as the Liberty County EMS Director.

Additional engineering tests were performed in Liberty County with LCEMS paramedic participation. Previous results indicated a severe limitation of cell phone coverage to major thoroughfares in Liberty County. A hybrid communications approach was designed, implemented, and tested by using two cellular providers: Sprint and Verizon. Field engineering tests have shown that this approach does enable a more broad coverage area and a greater number of cellular devices while reducing communication traffic collisions. All USB cellular phones have been upgraded to PCMCIA cellular modems integrated into a Linux based communications server.

A DREAMS administration station was installed at the Liberty County EMS offices in Daisetta, Texas. This system serves multiple purposes, most notably as the run record database repository enabling LCEMS staff to search and print archived run records. This system will enable LCEMS staff to search and print archived run records and, effectively, become a paperless office. It is serving as a training system for the LCEMS paramedics as they train their teams.

The Admin System will also serve as a communication hop for the Texas A&M development team to wirelessly link to the Digital EMS ambulance. The Admin System has been equipped with an 802.11 WiFi bridge. An external antenna was mounted outside the main office building to maximize the WiFi range.
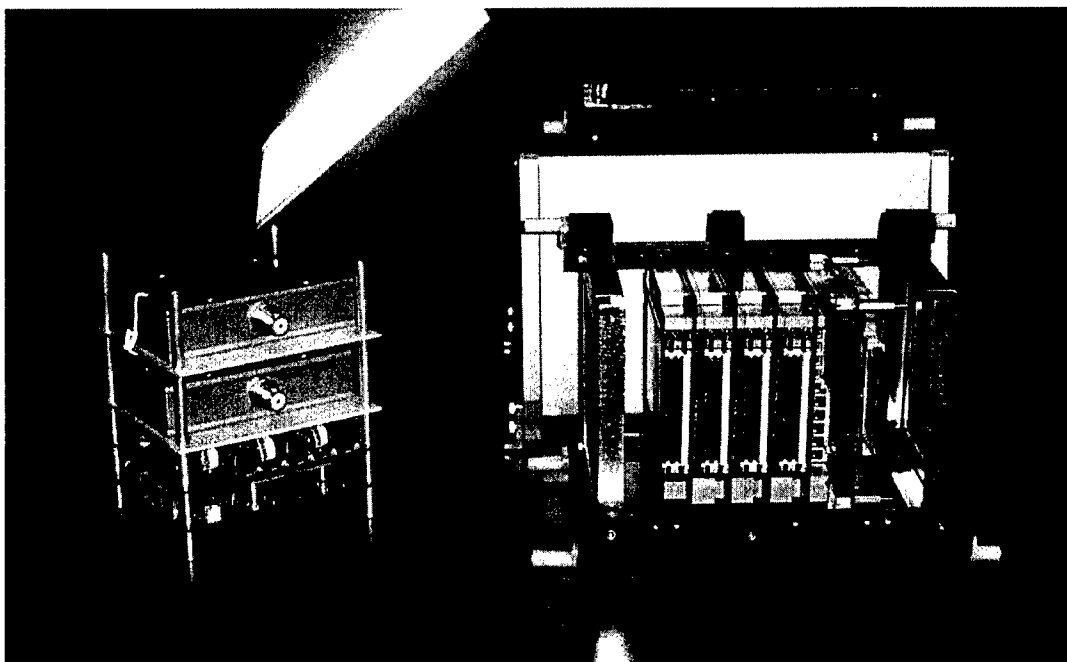
Photo 1: The PCMCIA cellular modems have replaced USB cellular phones.

Work has begun towards developing the required search capabilities of the Admin station and is expected to be completed early FY2005. Work is nearing completion on the capability of the ambulance to wirelessly transfer run record data to the Admin station.

Phase 0 engineering test runs of the Digital EMS ambulance were executed in Liberty County or the counties in the Brazos Valley. These tests were completed in March 2004 and solely utilized simulated patient and run record data. Each run constituted low-level testing of each Digital EMS component or module as well as the capture, transmission, reception, printing and display of the data. The final Liberty County Phase 1 volunteer patient runs were completed on July 16, 2004. Runs were carried out using the four developed scenario scripts and were distributed among the local, regional and distant testing categories:

> 31 of 20 required successful Local runs (< 20 min.)
> 21 of 15 required successful Regional runs (> 20 min., < 45 min.)
> 17 of 10 required successful Distant runs (> 45 min.)

Each patient run was documented according to the developed test plan. At the completion of each run, both the ambulance and physician run record databases were archived for after-action data transmission verification.

Frequently used LCEMS origin and destination addresses have been entered into the system as selection lists, thereby saving medic time when picking up or dropping off frequent patients. The cellular communication test client that measures the available bandwidth across geographic regions has been modified to utilize the new 3D cellular phones and modems. This utility

provides both individual device and aggregate bandwidth measurements geographically referenced by latitude and longitude, thus providing a means for assessing and visualizing areas with varying degrees of connectivity.

Software is currently being developed to enable this system to serve as the run record database repository enabling LCEMS staff to search and print archived run records. An automatic database archive capability was developed that will archive the database immediately upon disconnect, thereby ensuring that the paramedic and physician databases are successfully archived for database validation. The navigation server was also modified to output to a file the GPS coordinates of the ambulance during every run, thereby enabling a mapped route to be created for each run. Several members of the LCEMS team have become familiar with the Digital EMS interface through using the Admin System as a training tool.

We developed a data collection application that was used to scan barcodes of the entire set of services and supplies available on each Liberty County ambulance. This barcode listing was added to the barcode database, confirming that the Digital EMS ambulance will be capable of scanning and identifying all services and supplies on board the ambulance while in operation in Liberty County.

Planning and preparation was begun to begin Phase 2 testing in Liberty County during early FY2005.

Task 2: Digital Ambulance Systems Evaluation, Development and Integration
Meetings continued between A&M team members and Liberty County EMS. Minor additional enhancements were made based upon Liberty County EMS suggestions. Periodic support is continually provided to Liberty County EMS for their interactions with county officials, and other community leaders. Fairs and demonstrations are supported such as the Texas EMS Conference of 23 – 26 November 2003. These provide continual feedback to assure the project is continuing to meet the needs of the EMS community.

Signature capture and storage in the database has been completed. A polling module has also been developed to continuously inform the paramedic of the number of cellular devices that are currently available and transmitting. The LCEMS protocols have also been consolidated to a single file for easier management and distribution. Several functionalities have been added to the run record system such as field auto-completion and automatic specification of required treatment. A remote capability to re-build DREAMS databases has also been developed thereby simplifying remote installations.

A database manager utilizing a server-client methodology was developed to continuously synchronize data between a remote ambulance unit and its connected physicians' station. The manager is designed for minimal latency, with each run record field transmitted immediately upon entry. All run record data transmissions are sent at the highest priority thereby guaranteeing ordered delivery. If a device server becomes unresponsive, the connection manager will force a kill and restart of that server application. Likewise, if a remote system becomes unreachable the

connection manager will automatically un-register that system from all servers and re-register upon a successful "heartbeat" communication.

The University of Texas Health Science Center School of Health Information Sciences (SHIS) group performed a second round of interface and usability assessments that were completed on February 4, 2004. Each recommendation was rated by Texas A&M as to agreement and level of effort required to fix and assigned a developer point of contact. Significant progress was made towards completing the SHIS recommendations during the year but not all were completed.

The Phase 1 volunteer patient runs were very valuable in providing feedback on the Digital EMS system. All system usability comments and suggestions from the medics during the runs were recorded in the run documentation and assigned to the appropriate software developer for implementation. An ongoing goal was to have as many of the system improvements in place before the next set of volunteer runs began.
The printing system was completed and enables the medic to print out all run record forms. Additional forms were added to print the ECG and vitals data. This removes the requirement to have built-in printers on the medical monitors.

A new connection manager was integrated with an initial set of basic functions. Work continues towards migration of all servers to communicate with the connection manager upon initialization. The connection manager is responsible for handling all medic and physician server registration commands. The connection manager contributes robustness to the system by monitoring server response through heartbeat messages and restarting stalled servers as necessary.

The video server has had several significant improvements. The video compression library now utilizes a de-interlacing filter that results in video frames with less motion artifacts and higher compression ratios. The video quality was also improved by changing the color format of the decompressed video. The video server now automatically anticipates frame rate adjustments when the Physician changes the video quality. If the Physician chooses a higher quality, the video frame rate is adjusted to utilize the same amount of communications bandwidth.

The Paramedic system was improved based upon feedback from the Liberty County paramedics. The medics can now enter multiple medications simultaneously, enabling an immediate capture of the medication and timestamp and the ability to edit amounts and route at a later time. The thermal printer was replaced by a more reliable, quicker and archive-capable inkjet printer. The medics can now edit logged skin observations as well.

Nearly all recommended improvements have been successfully incorporated into the Digital EMS system and work continues to complete the entire list.

Similarly, testing revealed that the system messages module can fail to show lower priority messages while higher priority messages are being displayed. The module was redesigned to allow prioritized and timed alerting of messages, guaranteeing that all messages will be displayed in a prioritized order for a duration appropriate to their priority.

During Phase 1 testing Liberty County requested a "local" audio capability enabling two-way audio just between the driver and the paramedic in the back so they don't have to yell back and forth through the pass-through window. This feature has been implemented, giving each medic the option of communicating only to their partner or to the remote physician as well.

The map creation routine now starts in its own separate thread, thereby enabling the user to continue interacting with the system the map is initializing. The camera control server was redesigned to support a driver architecture and upgraded to use Panasonic's latest camera protocol. The graphical user interface was improved to make it more robust. The printing module is currently being analyzed in depth to ensure accurate presentation of every possible item and grouping of data. Work will be completed in FY2005 in time stamping and storing all GPS coordinates and audio segments in the database for post-run analysis.

Hardware modifications to capitalize on USB peripherals and devices have begun. This reduces the wiring and cabling required as well as reduces the required interfaces to be supported in the third generation hardware. Most notable of these for the moment is the development of a video and high resolution, still image camera. This hand held camera is being developed specifically to meet the requirements suggested by the Air Force but has significant utility in the civilian version of the ambulance. The resolution of the video stream will be a significant improvement over the current video capabilities. The high-resolution still images will exceed 1 mega-pixel. This camera and its USB interface are also driving the development of video server modules (hardware and software drivers) to enhance the architectural flexibility to support any of a wide range of cameras and interfaces

Task 3: Hospital-Side Systems Evaluation, Development and Integration with Digital Ambulance

The software testbed located at IBT in Houston was updated with the latest version of the DREAMS software and hardware as required. This is used to support interface and usability assessments by the University of Texas Health Science Center School of Health Information Sciences (SHIS) group. The testbed is also setup to be the primary physicians' station during various stages of field testing.

Physician workstation hardware was defined for support of the Liberty County EMS and Hermann Hospital. This equipment was configured and installed to support field testing. During the first introduction of the Digital EMS system to Hermann Hospital physicians, a request was made for a capability to send textual messages back and forth between the ambulance and the physician workstation. This "text messaging" capability was added and follows conventional client/server model used throughout the system. It behaves much like current instant messaging systems in that received messages will automatically cause the dialog to appear. The dialog can be toggled

on/off and is fully resizable. This capability allows a written and time stamped record of all dialog and intervention orders for after-action analysis to be stored with the current run record. After evaluating the usage of the physician's telestration pen tablet, it became evident that

drawing using a separate tablet device is not as intuitive as using a mouse. As such, telestration has now been fully integrated into the video client, thereby allowing the physician to simply use the mouse to draw and clear lines on the video images. Additionally, this integration reduced the number of required devices on the physician station. Physician telestration lines are stored by camera and can be drawn in any of six colors. The physician can toggle between telestration mode and region of interest (ROI) mode, thereby allowing both video capabilities through single mouse interaction on the video panel.

The camera control server was also modified to provide more robust remote Physician control. Depending on network traffic and routing the speed at which the start and stop camera movement commands could vary greatly, thereby introducing randomness into all camera movement durations and leading to unintended results. The Physician now sends over a single movement duration specifying the direction and length of time to move in that direction, preventing wayward results.

The navigation system has been improved to send the Physician the ambulance destination coordinates. The Physician not only receives the distance and estimated time of arrival, but also sees the destination marked on the map. The printing system has been added to the Physician station, enabling the Physician to print out run record pages and ECG waveforms at any time.

The text messaging system was improved to support color indicators of the sender. Messages sent by the user are displayed in a subtle blue color while messages from the remote person are displayed in bright white, thereby improving the user's ability to "view" the conversation. All dialog messages and intervention orders are time stamped and stored in the database for post-run analysis.

Bio-logon using finger print reader equipment was selected and incorporated into the physicians workstation. This allows physician registration and logon quickly and without yet another password to be forgotten.

Task 4: Develop Communications Infrastructure to Support Hospital-Side and Remote-Site Communications Systems
The firewall infrastructure at the Texas A&M Institute of Biosciences and Technology building in Houston was modified for security reasons to filter out User Datagram Protocol (UDP) packet fragments. This became a problem when large row and table data was being transmitted to the physician station. Packets larger than the maximum transmission unit size were split into fragments and, hence, filtered out by the firewall. The central datagram socket communication protocol was modified to support automatic packet splitting, thereby preventing any fragmentation.

The Motorola XTS5000 radios were provided to the project by AFMESA for development and to support the evaluations by the Air Force. These radios will be coupled with modems developed by Worcester Polytechnic Institute (WPI) for data communication over simplex radio

audio channels in FY2005. The radios have been tested to a limited degree and are excellent radio systems with about a 5-mile line of sight range.

The Ku band satellite antenna terminal was installed on the Interact Demonstration ambulance and tested. Reconfiguration of the satellite system for use in an application setting rather than a development environment was completed. Dr. Robert Arnold traveled to NRL on several occasions to participate in the continued testing of the mobile satellite terminal. During testing of the cross-polarization characteristics of the antenna using a PanAmSat satellite coincidently demanding maximum rotation (polarization) of the feed horn, a bug in the vendor supplied control firmware was discovered. This bug would have no effect during normal operation of the terminal, but would continually be an irritant during further certification and testing. The vendor revised the firmware and the unit now works correctly.
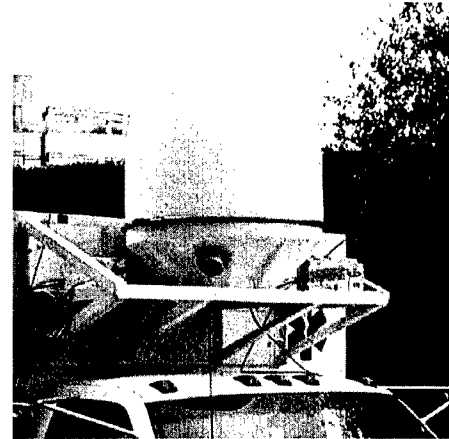
Type Approval for our Ku-band 0.6 meter mobile terminal has been granted allowing access to PanAmSat satellites. This approval is very unusual in that it is for the entire hardware configuration as a system instead of the normal approval for just the uplink portion. This is the only such approval for a mobile, terrestrial antenna ever done by PanAmSat.

The ground station terminal for the DREAMS project at the IBT building in the Texas Medical Center has been fully installed. PanAmSat has certified this base station terminal for use with any of its satellites in multiple modes.

Testing will continue in both stationary and mobile conditions. Initial paper studies and design work was done by NRL to provide further details toward the development of the low profile Ku / Ka band mobile satellite terminal that meets the ambulance height restrictions, is less complex, and is significantly less expensive to manufacture.
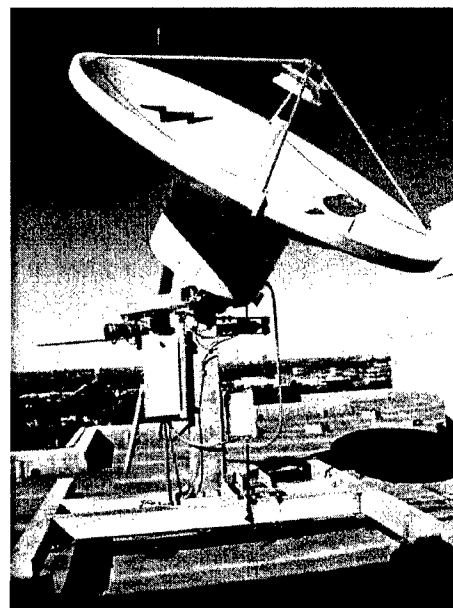
Development of new cellular modems is continuing in industry and as they become available on the market, they are being tested for application in the DREAMS system. In this regard, several Cingular GC82 "Edge" cellular modem cards were acquired on loan and tested. While their specifications and marketing literature indicated a good fit for this application, our use and tests indicate their upstream bandwidth is too limited and the communication channel too unreliable.

Task 5: Integrate New Technologies into the Existing System Architecture; Enhance System Functionality and Support Upgrades

Phase 1 testing revealed a difficulty of comprehension in audio transmissions between the paramedic and physician stations. After much research and testing, it was determined that the lack of audio clarity was the result of the poor compression library and the added compression introduced by the paramedic's wireless encrypted headset. A more robust compression library was integrated that not only improved audio clarity but also enabled variable length compression, enabling higher compression and smaller audio transmissions during moments of pause or silence.

The 12-lead ECG device server was successfully modified to follow the parsing deviations present from the manufacturer's published parsing protocol. The device now receives, transmits and displays correct waveform data that has been verified using the simulator and comparing against the manufacturer's supplied software. Any number of 12-lead ECG waveforms can be logged per patient and the user can step through each of the time and date stamped waveforms. Paramedic and physician printing of the 12-lead waveforms are expected to be completed early in FY2005.

An "Emergency" button was added to the industrial sealed keyboard to automatically display the "Connect to Physician" dialog, thereby enabling a quick way for medics to contact a trauma physician. The vitals server was upgraded to facilitate an increased robustness during various operational situations.

Data transmission between the ambulance and the physician has been improved. A data compression library was integrated, enabling the transmission of compressed run record data at greatly reduced bandwidth requirements. A data field update method was created that enabled a change to be individually sent instead of using the more bandwidth-consuming row update method containing all member table fields. Data updates are now specified using a one-byte column index instead of multi-byte column names, thereby reducing bandwidth. Record indexes have been abstracted so as to prevent remote potential conflicts among multiple remote units connected to the same physician. The Digital EMS table structure has been refined to isolate run record data from patient data. This enables a "one to many" relationship which, if desired, could associate multiple patients with a single run record. This capability could save typing on the part of the paramedic and will be analyzed to verify usability and proper operation. A standalone physician name server has also been developed to store and transmit all current physician workstation IP addresses to all remote units.

The navigation system was enhanced to better support the Driver and Paramedic stations on the ambulance. When either station specifies a navigation destination on the Map panel, the route information is transmitted and computed on the other station as well. This enables both stations to view the route and arrival information when either station enters a destination.

The speed and performance of the printing system has also been improved. All aspects of the system, including PDF creation, previewing and printing, are now more efficient and immediate.

Testing revealed Linux problems with the point-to-point protocol (PPP) daemon and the chat system in which Linux will, over time, leave modems in an unusable state. The Intelligent Communication Manager (ICM) was successfully upgraded to a Linux Gentoo distribution with a version 2.6.x kernel. Version 1.3 of the ICM software was completed and allows for multiplexing IP addresses by the physician. The Intelligent Communication Manager (ICM) software version 2.0 introduced an entirely different transmission framework and usage modifications. Testing revealed several significant issues with version 2.0 and rendered it unusable. These problems have been resolved. This version has been thoroughly tested and has been certified as the field release ICM version. The ICM now has the capability to automatically initiate dial-up and hang-up procedures without any manual interaction required. More significantly, the ICM now enables the physicians' workstation to be connected to two or more remote units simultaneously. Modifications to the connection module and the underlying datagram socket mechanism were made to accommodate the version 2.0 functionality.

ICM version 2.1 is currently in development and will include statistical feedback and more advanced load balancing across multiplexed interfaces. ICM version 2.2 is also currently in development and will include more advanced interface configuration, an expanded rules engine and support for heterogeneous channel selection based on precedence and other performance factors.

The video system software underwent several significant modifications to further automate operation. A potential problem was identified in which the physician could overload the cellular communication system by choosing both high video quality and fast video rate. The physician now only chooses one of twelve quality levels and the frame rate adjusts automatically based upon available bandwidth. The video server also caches the camera thumbnail images and sends them to the physician immediately upon connection. The video throughput monitoring system was improved to enable real-time reporting of received frames, thereby reducing the latency in adjusting to low or high bandwidth changes. A video splitter module was added to the video server that enables video frames to be split into 1500 byte segments so that they will fit under the MTU limit. Prior to the video splitter, video frames were fragmented by the lower-level phone socket thereby forcing all fragments of a video frame to be transmitted over the same modem.

The USAF requested the integration of an IP based portable ventilator prior to the field testing of the HMMWV ambulance. A server and test client has been developed to integrate this device and was demonstrated to several active duty USAF physicians in December 2003

The next generations of computer hardware design were begun. These versions are intended to occupy half the volume with equal or greater computing power. They are all DC powered and do not require a UPS. They are expected to weigh 50% of previous versions and are designed to require less external cooling. The intent is to build as much of this equipment into ambulance dead space as possible. Form and fit models indicated that these goals are achievable. Digital video/audio recorders are integrated in this design. All disks are removable and most system components are modular. Functional prototypes were built and are under test.

Additional wireless vendors and cards were tested and added to the system. Multiple cards operating on each of 2 separate networks provides redundancy for adequate bandwidth as well as infrastructure redundancy to insure network coverage by using multiple network systems. This equipment is now being repackaged into significantly smaller and more rugged cases that were designed during this period. Enhancements and testing of this system will continue.

New versions of the specialized "Cell manager" hardware were designed, built, and tested. It was determined that two small chassis instead of one larger one gives better temperature tolerance and component integration. The GPS system is now integrated in the Cell manager hardware with a significant savings in weight and space.

Efforts have begun to employ new interfaces for peripheral equipment and medical devices. The most recent generation of devices commonly uses the Universal Serial Bus (USB), wireless and Ethernet in place of older serial RS232 interfaces. In addition, cameras are readily available with USB and network interfaces, eliminating the need for frame grabber interface cards (which are expensive and frequently are the ultimate performance limiter). Next implementations of the ambulance hardware will use the handheld USB camera developed at the request of AFMESA. They will also use the network version of the current full motion control camera. The architectural adaptation of the system to meet these needs uses a video server module that abstracts all the camera control and image/video interface issues away from the basic application. The video server module will handle all existing analog cameras as well. Thus all issues regarding images and video will be located in a single module and provide a single uniform interface to the rest of the system.

Task 6: Initiate Field Testing of Digital EMS System(s)
Field-testing results on the efficacy of the PCMCIA cellular modems showed that the utilization of dual cellular providers offers a larger area of connectivity. Mobile and static testing were performed on the satellite terminals on multiple occasions. Tests were conducted from the vehicle to IBT and from the vehicle to the Naval Research Laboratory in Washington, D.C.

In the third quarter, representatives from Texas A&M and UT Houston flew to Laredo, Texas to meet with officials in preparation for a second deployment. Discussion topics included logistics, communications and the need for this technology in an otherwise remote area. Legal documents to support this activity are expected to be completed early in FY2005.

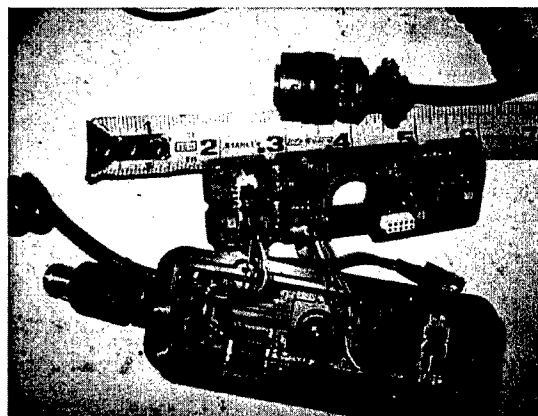03/30/2004

Liberty County Phase I Testing

The Phase 1 volunteer patient runs in Liberty County were completed this year. Patient runs were documented according to the developed test plan. At the completion of each run, both the ambulance and physician run record databases were archived for after-action data transmission verification. The acquired data was then compared for accuracy in transmission. Any discrepancies were examined and the cause determined and corrected. The required amount of successful runs in the three distance categories were met and work is now focused toward supporting Phase 2 testing in Liberty County.

Task 7: Design and Develop Initial Prototype of Digital EM System for Military Use
The HMMWV computer system was redesigned to one/third the volume and about one/third the weight. Weight and size has continued to shrink during this year's efforts. These computers are all DC and consume significantly less power. The requirement for AC power has been eliminated for all DREAMS specific equipment but the converter will remain for use by military personnel. Prototype development has continued and evolved. It is now feasible to use a very similar design and package for the HMMWV, certain civilian vehicles and the next generation DTS.

Work continued on the development of better video transmission for use by emergency service personnel. This includes the design and testing of very small, high gain patch antennae that can be carried and simply deployed by victim recovery personnel. Tests indicate that the design is viable and can be cheaply reproduced. Variations of this will constitute wireless repeaters to extend the "on-line" search distance.

In order to support the U.S. Air Force requirements, work was done toward developing a 1.1 mega-pixel still image capture, transfer and display capability. A prototype still image transfer method was developed that achieves the requirements. Once a digital image is taken and the camera plugged into the system, the image is displayed on the paramedic station for approval or rejection. If rejected, the image is simply deleted off the camera. If accepted, the image is then transmitted and displayed in a window on all paramedic and physician stations. This capability supports the quick capture, acceptance, transfer and display of 1.1 mega-pixel images. Work continued on the development of the video control system using PC104+ components. Additional enhancements for video support and efficiency were discussed and planned for future development.

Work is ongoing to support the integration of the Motorola XTS 5000 VHF radios. As currently developed by Dr. Michalson, the Ethernet modem interfaces to the radios should be able to integrate seamlessly in a manner similar to the Intelligent Communications Manager. A cyclic redundancy check (CRC) capability was integrated into the datagram socket manager, thereby validating transmissions over what could be a lossy and non-validated medium. Delays in modem development at Wooster Polytech have moved any testing into FY2005.

Development of a smaller, lighter Deployable Telemedicine System (DTS) has continued. New cases and chassis' have been specified and ordered. Design work on a universal power module was initiated and is well advanced. This module will be housed in a separate Hardigg case and weight less than 136 pounds. It uses 12 or 24 volt DC or 110, 220, or 240 AC as input power. Output is multiple streams of 24v DC to run system components. Internal batteries allow the system to survive low voltage (AC brownout) disturbances or the changing of externally connected power sources. Internal batteries are automatically recharged by available external power sources. All output circuits are designed to be surge suppressed and have Ground Fault Detection Interruption (GFDI) protection.

The revision of this package will also enhance the next generation of equipment for the civilian ambulance and provide a package of equipment for "vehicles of opportunity" as required by the Air Force for its applications.

Task 8: Participate in Military Tests, Concept Evaluations and Exercises
A DREAMS overview presentation and demonstration of HMMWV, DTS, and assorted DREAMS developed technology was given on 17 December to the General staff of former Air Force Surgeon General P. K. Carlton. Significant interest was shown in DREAMS technology being used at the Air Force medical training facility in Cleveland, Ohio. The PC104+ based

portable computer systems were identified as possible solutions to air evac monitoring problems encountered in Iraq. Further details were requested as the products develop.

The Air Force demonstration, originally scheduled for March, was delayed at Air Force request to allow time to more fully determine their communications strategy. It is expected that the demonstration will use the Wooster Polytech modem coupled with Motorola 5000 VHF radios. A preliminary meeting was held with Dr. Michalson, from Wooster Polytech, and a timetable was set.

The DREAMS Project participated in an initial concept evaluation exercise with AFMESA at Ft. Detrick, Maryland during the week of August 18th 2004. This exercise was to familiarize the AFMESA team with the capabilities and possibilities of the DREAMS HMMWV. On this basis work is being done with AFMESA toward two additional demonstrations including a complete formal Military Utility Assessment (MUA). A MUA is a preliminary step in the development of an overall procurement plan. This exercise was very successful and attracted the attention of the Surgeon General of the Air Force, among others.

Task 9: Evaluate and Pursue Opportunities to Develop and Implement the Digital EMS System in Disaster Response Scenarios

The DREAMS group is continuing to investigate more powerful, less intrusive camera solutions. The ability to extend transmission range by using small, easily deployed RF repeaters continued to be investigated. The small, high gain patch antennae developed previously was tested further and a novel packaging scheme was examined. Tests indicated that the design is viable and can be cheaply reproduced. Variations of this will constitute wireless repeaters to extend the "on-line" search distance. The prototype repeater design was enhanced and was built for bench test. Gain was acceptable but the size has to be reduced. Work on this device has been suspended due to other project requirements.

Preliminary analyses were done to determine how portable, how small, and how waterproof the DREAMS communications system could be made. This work will be continued later and possibly transferred to additional research projects for use on small format HAS-MAT and victim retrieval vehicles.

Work continued on developing a significantly smaller DREAMS package to be used on smaller, more agile vehicles as well as to be "built in" in larger, traditional vehicles. These endeavors are aimed to not only save space but also reduce power consumption. This work contributes to space savings in civilian and military ambulances.

The initial development of a revised, smaller, lighter Deployable Telemedicine System (DTS) has begun. This design has modularized several aspects of the DTS eg the power system is now capable of running from any reasonable power source from 12 VDC thru 240VAC without user intervention. All internal components are powered from an internal 24VDC power bus thus eliminating multiple 110 VAC to DC power modules used in the previous DTS. This raises the efficiency and reduces the power backup battery size further reducing size and weight. The

modules developed for the new DTS will also be used in the HMMWV, the civilian ambulances and the package for vehicles of opportunity required by the Air Force.

Task 10: Work with University of Texas Health Science Center to Enhance Current Technologies within the Digital EMS Vehicle and Associated Hospital Systems
Evaluation of emerging medical technology and portable equipment continued through this year. Work continues in evaluating other wireless devices and methodologies that may be applicable to the project needs.

An evaluation of a fingerprint scanner device versus iris recognition device was conducted to meet the physician login requirement. The team reviewed the evaluation and the fingerprint scanner device was chosen for integration. Evaluation continues on wireless devices and methodologies that may be applicable to the project needs.

It is extremely beneficial to minimize the obstruction of the paramedics on the ambulance. One example is to reduce the wiring required to hook the Propaq vital signs monitor to the DREAMS computer system. The UTHSCH has ordered a wireless Propaq CS vital signs monitor. It has been confirmed that the device protocol mirrors the wired version. Early in FY2005 work will be done to determine the feasibility and level of integration effort required to support the wireless Propaq.

Task 11: Enhance the Existing Digital EMS System to Accommodate Additional Functionality
Engineering testing was done to evaluate the efficacy of the transmission of the ECG waveform data. Due to the unreliable and dynamic state of wireless communications, many of the small data packets were never received or arrived in an irregular sequence. As a result, the vitals module was modified to buffer and transmit waveform data in 8-second increments. This modification has greatly increased the ability of the waveform to be interpreted. Each waveform image now contains a visual time stamp and is capable of being stored in the database. The physician now has the ability to enable and specify the interval at which patient blood pressure can automatically be taken.
A text messaging system was developed and provides a backup communication mechanism between EMTs and physicians during low bandwidth situations. All text messages are stored chronologically in the database and archived with the run record for after-action review.

Research continued to enhance the quality and extend the reach of portable video delivered from the EMT to the ambulance. As indicated earlier, new enhanced cameras were investigated, as well as improved wireless communications links having longer range with the possible use of repeaters.

Significant time was spent early this year on the customization of the new 14-foot ambulance design from Frazer. There were changes to the standard ambulance design required to support

DREAMS. Equipment and mountings for that equipment had to be slightly modified due to evolutions in Frazer's basic design. A design for removable computer frames for the new ambulances had to be done in order to support various generations of systems. Significant work was done in implementing these designs in the 14 foot Frazier ambulances to prepare them for internal wiring upgrades, other equipment and the specialized, research optimized, computer-mounting compartment.

The Interact ambulance was analyzed for mounting of the satellite dome onto the front of the box over the cab. The parts and design were completed and the antenna has been installed in the Interact vehicle. The rest of the terminal equipment was installed in a mini 19" rack inside the box.

New DREAMS subsystems are based on Linux platforms. The Future Combat System is targeting the Linux operating system on the Apple G5 PowerPC platform. In order to accommodate a variety of deployments, DREAMS software has to move away from the Windows-only motion wavelet compression algorithm. An in-depth evaluation of video coding options was performed to determine viable compression libraries that are compatible with various platforms and operating systems. The evaluation included performance ratings as well as feature set. One library has been identified as being a great replacement and integration evaluation of this library may begin in early FY2005.

Task 12: Integrate New Technologies for Inclusion in the Digital EMS Vehicle to Support Additional Medical Functionality for Trauma Care at Remote and Hospital Sites
The U.S. Air Force requested the IMPACT Instrumentation, Inc. network based portable ventilator for integration in DREAMS. . This device is a prototype and was provided by the vender. The server and test client were successfully developed and tested. The device server receives and parses data from the network based portable ventilator. A ventilator interface panel was developed and client integration with the panel was completed. In preparation for the U.S. Air Force demonstration done August 2004, support for the network based portable ventilator was integrated into the paramedic and physician applications. Both the paramedic and physician can view the numeric and waveform data from the device. If desired, the ability to control settings and receive alarms remotely can be enabled.

An on-going dialog was established with Midmark with the objective the integration of their portable 12-lead ECG device into DREAMS.

The discussions were successful and this device was integrated and tested in the field for the first time with mixed results. The data stream from the device did not follow the manufacturer's published protocol. The manufacturer provided sample code, which offered insight on the parsing abnormalities and a 12-lead ECG simulator was also purchased in order to verify correct operation of the device, our server, and the client waveform display. The 12-lead panel was modified successfully to show all twelve waveforms in a 3x4 2-second format with an 8-second rhythm lead. ER physician input was used to establish best practice presentation of the data.

Task 13: Integrate new US Army, NASA and DARPA Technologies Such as Medic-CAM and WARP into the Existing System to Enhance System Functionality
The existing Medicams were returned to TATRC. All support or demonstration work related to the Medicam has been stopped. Any activity in remote camera technology will be confined to DREAMS developed devices.

A low level of activity at a low priority continued during this year. Heads-up displays, communications mechanisms and miniature handheld user interfaces continued to be investigated but no outstanding examples were found

Task 14: Enhance Existing Infrastructure for Supporting a Network of Multiple Digital EMS Vehicles and Hospital Systems in an Integrated Environment
The system communication architecture was designed to support multiple simultaneous vehicles and users. As systems such as the Liberty County vehicle, the HMMWV and the mobile satellite test vehicle become available in the same geographic area, testing this capability is possible. The Intelligent Communication Manager software was continually upgraded to enhance this ability.

The WiFi network topology of the entire system was improved to support multiple Digital EMS ambulance vehicles at the LCEMS main office as well as destination hospitals. This upgrade required the installation of a second WiFi access point in the ambulance and reprogramming of all WiFi components in order to overcome design limitations in commercial off-the-shelf products. Security settings were set that restrict communication traffic exclusively to Digital EMS systems. A WiFi network bridge with external antenna was installed at the Liberty County main office. A T1 network connection back to the IBT was ordered. Once the T1 access is installed in FY2005, Texas A&M developers will be able to communicate remotely with one or more Digital EMS ambulance vehicles in the field.

Task 15: Develop and Test a Prototype Digital EMS Vehicle in Diverse Urban and Rural Technologies for Evaluation and Performance Analysis of Integrated Digital Technologies
Communications testing continued within Brazos County and Liberty County, inside and outside the city limits. Tests focused on communications as well as system usability over the unreliable cellular network. TAMUS personnel continued to work toward the initial Liberty County deployment.

Two new ambulances were delivered in the third quarter. These designs include the computer systems and support equipment being built into the ambulance structure instead of using space traditionally given to the paramedics. After delivery, work continued on the 14 foot Frazier ambulances to prepare them for internal wiring upgrades, other equipment, and the specialized, research optimized, computer-mounting compartment. Some of the hardware components were ordered to install into the two new units but installation was delayed due to limited personnel and design evolution. Work on these units has benefited from knowledge gained in Phase I testing and will resume after more information is gained from the Phase II testing in early FY2005. It had been originally planned to outfit Liberty County hospital with a DTS based system. Negotiations with the Liberty County hospital were suspended awaiting the resolution of internal hospital issues.

The DREAMS Digital EMS ambulance was exhibited as part of the Internet2 conference in Austin, Texas, on September 28-29, 2004. Throughout the two-day conference the ambulance was connected over 3G cellular to the DTS running as the physician station in the exhibit hall. The incredible quality and rate of the video stream was an authentication of the varying bandwidth based upon infrastructure. Performance over the downtown Austin cellular network demonstrated a 400% higher bandwidth capacity than in suburb and rural locations. While some difference was expected, the degree of disparity was unanticipated.

Task 16: Develop Methodologies for Using New Local, State and National Network Infrastructures for Providing the Digital EMS Vehicle with High Speed Terrestrial Connectivity to the Hospital Node
During 2004, many activities in this area were in progress but there is no significant activity to report at this time. It is expected that mid-FY2005 will bring a number of opportunities for DREAMS collaboration with or utilization of emerging network projects.

Task 17: Publish Findings and Results in Appropriate Conference Proceedings and Journals and Demonstrate Capabilities of the Digital EMS Ambulance
- October 15 – 17, 2003 - Trinity Valley Exposition, Liberty, TX
- November 23 – 26, 2003 - Texas EMS Conference, San Antonio, TX
- December 18, 2003 - Demo for TAMU Director of Homeland Security
- February 19, 2004 - Demo TAMU Director of Homeland Security/Northrop Grumman

- March 23, 2004 – Demo to Impact Instrumentation/NASA Wyle Labs
- April 6, 2004 - State of Texas budget staff
- April 7, 2004 - Los Angeles Fire Chief
- April 8, 2004 – Smart Medical Technologies Summit 2004, Houston, TX
- May 2-4, 2004 – American Telemedicine Association, Tampa, FL
- June 21, 2004 - TX US Senator Cornyn staffer
- July 14, 2004 – Demo to Dr. Charles Galloway, Director DOD Chem/Bio
- August 10, 2004 – PLR in Frederick, MD
- August 15–18, 2004 - AFMESA Initial Concept Evaluation
- September 26–28, 2004 - Internet2 meeting in Austin, TX

The Texas A&M University System Detection and Remediation of Chemical and Biological Threat Agents Program

Task 1: Determination of the Catalytic Limits for the Existing Organophosphate (OP) Hydrolases

The well-understood, enzyme-based catalyst (OP-hydrolase) that can be used to detect and decontaminate chemical warfare neurotoxins including G-type and V-type agents has been characterized in detail during the first four years of this project. This year the Raushel laboratory has concentrated its efforts at the selection of new mutants of phosphotriesterase (PTE aka OPH) that have enhanced catalytic activity toward the hydrolysis of the most toxic stereoisomers of GD. Single substitution libraries were constructed for position His-254, His-257, Leu-271, Ser-308, Tyr-309, Met-317, Gly-60, and Ile-106. Sample randomness was confirmed by sequencing selected colonies from each library. Enhanced mutants were obtained for H254R, H257Y, H257W, S308A, Y309F, and G60T. Double substitution libraries were constructed in the context of H254X/H257X, H254X/H257X/L303T and G60A/H254X/H257X. The enhanced mutants obtained from the initial screening efforts included H254Q/H257F, H254G/H257W, H254G/H257R and H254R/H257A. These mutants will be tested with sarin and soman since they are able to hydrolyze the most toxic analogs of soman approximately 100 times faster than the wild type enzyme.
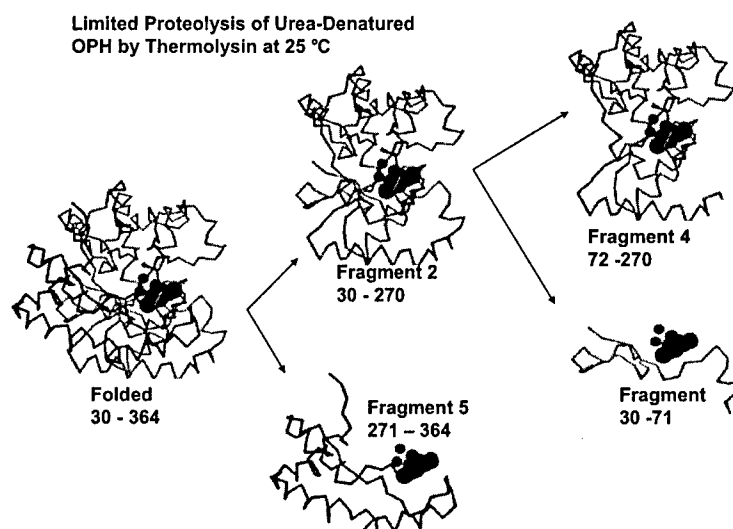
Task 2: Investigation of Mutations of Individual Residues and Creation of Rational Combinations

Several double and triple variants have been created (254X/257X/---) which demonstrated the significance of a series of aromatic side chains in active site adaptability, resulting in enzymes with increased activity against the P-S bond of VX (as demonstrated by the analog Demeton-S). In addition to the changes observed in the substrate profile, the 254/257 variants are considerably less stable (see 04 Third Quarter Report). Believing that the RL mutation moved the substrate profile in the desired direction (greater activity with the P-S bond of VX), the objective of the experiments reported here is to create an enzyme with the substrate profile of RL, but the

stability of the WT OPH. Genetic changes at position 254 and 257 were replaced individually and in concert, the substrate profile was dramatically altered in often opposing directions [PX, paraoxon; DFP, diisopropylfluorophosphonate (a soman and sarin analog); NPPMP, and D-S, demeton-S (a VX analog)]. Evaluation of this variant series led to the engineering of a double mutation replacing the 257 residues with a phenylalanine. This significantly increased the stability as measured by proteolytic sensitivity, as the strength of the stacking interactions increased, so did the protease resistance. This enzyme was able to balance an increased stability over any enzyme previously created, and maintain an intermediate activity with the target substrate D-S.

Task 3: Investigation of Combinatorial Mutagenesis of Amino Acids Affecting the Active Site of the Enzyme

This study is designed to rationally evolve new enzymes that balance the more effective catalytic specificities developed by site-directed mutation with enzymes designed for enhanced stability. The application of enzymes in the detection and destruction of chemical warfare agents requires an enzyme of exceptional stability as well as catalytic specificity. The studies described here, ongoing from previous quarters, begin to elucidate the unfolding pathway of organophosphate hydrolase, thus facilitating the intelligent design of variants with increased stability. The structural implications of this pattern are shown below, with the active site defined by the

Limited Proteolysis of Urea-Denatured OPH by Thermolysin at 25 °C

Fragment 2
30 - 270

Fragment 4
72 -270

Folded
30 - 364

Fragment 5
271 – 364

Fragment
30 -71

blue space-filled model of the inhibitor DE4MBP. The hydrolytic capability of the fragments remains to be determined. By carefully defining the unfolding profile of the enzyme under different conditions, those regions of the protein which initiate the process can be defined, and then engineered for stabilizing interactions.

Task 4: Introduce the Best Currently Existing Enzymes into Existing Biosensor Detectors

A variety of different enzyme-surface scaffolds have been studied to try to develop a robust optical biosensor that can discriminate between different chemical warfare agents and their more benign, but readily available, structurally-related agricultural pesticides. To date, the most successful approach to attaching OPH to form reactive surface platforms has been the layering of

avidin, biotin, enzyme or BSA, and CNF (ABOC) in that order. Starting fluorescent signals ranged from 8000 to 22500 averaging around 18000. There was significant signal decay initially which stabilized after 30% of the signal is lost. In order to better activate the surface different plasma gases were tried and Argon was used for cleaning all particles off the surface of the biosensor wave guide; oxygen was used to increase wetability of the polystyrene. Oxygen may bind to polystyrene and create a reactive group that provides better binding. In order to evaluate the response of the existing sensor with alternative substrates, a variety of OP pesticides were selected for testing purposes. The detection limits were Acephate = 2uM, Dichlorvos = 5uM, and Demeton-O methyl = 5uM

## Task 5: Selection and Purification of Mutants with Specifically Desired Catalytic Characteristics

Previous studies in our laboratory have shown that modifications of amino acid residues surrounding the active site of OPH have had distinct effects on catalytic activity and substrate specificity. Based on these results, other changes were proposed that might further improve the positioning of larger, hydrophobic substrates within the active site by either increasing the size of the binding pocket or by introducing a larger hydrophobic side chain to accommodate like groups. These prospects were critically evaluated on the basis of the known crystallographic structures of various forms of OPH. Catalytic rates, either k_{cat} or units/mg, were determined by performing enzymatic assays on changing (k_{cat} for pure modified OPH) or saturating (units/mg for partially purified OPH) concentrations of each substrate of nine modified enzymes, four showed an increase in the hydrolysis of demetonS compared to the wild type.
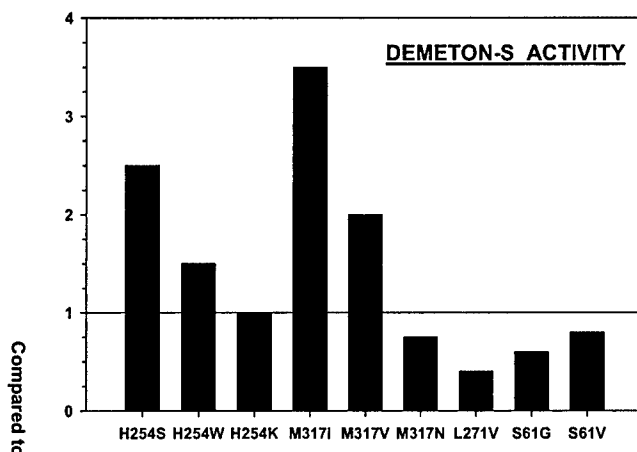
Task 6: Introduction of the New Genetically Engineered Proteins into New Biological/Chemical Sensors

Sub-task 1. Enzyme-based Biosensors for Organophosphate Detection – Technology Transfer to Analyte 2000

One of the common mechanism by which proteins are incorporated onto sensor surfaces is cross linking with amine reactive reagents. The most significant factors affecting an amine's reactivity are its class and its basicity. Virtually all proteins have lysine residues, and liphatic amines such as lysine's Є-amino group are moderately basic and reactive with most acylating reagents. There are 6 lysines in OPH, so incorporation of OPH onto sensor surfaces using amine reactive reagents results in OPH being conjugated in a variety of orientations. One lysine in particular, K175, is problematic as it is located near the active site (shown in green in the figure below) and is the only solvent accessible lysine on this face of the enzyme. Surface attachment via this residue could result a "face down" attachment resulting in the active site being inaccessible to substrate.

Surface Lysines

K175
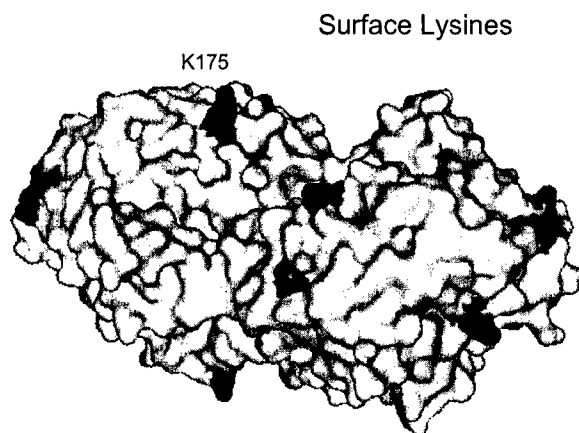
Site directed mutagenesis was used to change this lysine to an alanine. Evaluation of the activity of this variant verified that the activity was not significantly affected in the free enzyme. nitial studies incorporating this variant onto sensor surfaces revealed that: 1) with identical attachment procedures, about a third the amount of K175 was surface attached as compared with WT (3 nM vs 1.3 nM), and 2) in spite of this, the K175 surface had similar, or slightly greater activity. This work is ongoing, but it is important to note that conditions can be identified which will increase the amount of K175 of the sensor surface, which should result in a significant increase in surface activity.

Sub-task 2. Attachment Chemistries on waveguides and decoration .
Thus far, the most successful approach is the subsequent layering of avidin, biotin, enzyme or BSA and CNF (ABOC) in that order. Starting signals ranged from 8000 to 22500 averaging around 18000. Initially there is a significant signal decay initially which stabilizes after 30% of the signal is lost. Another attachment chemistry was attempted in which the surface of the waveguide was activated with silane followed by incubation with gluteraldehyde. This did not work therefore instead of silane nitrogen plasma was used to activate the surface followed by incubation with gluteraldehyde. The signal was not as high as was obtained with ABOC but it was reasonable and around 7000-8000 but very little decay was also observed. Another approach adapted to reduce signal decay was to switch off the laser in between injection of samples.

Comparison of gluteraldehyde and ABOC chemistries indicated that still ABOC was a better decoration technique than gluteraldehyde.


Task 7: Introduction of the Genetically Designed Enzymes into Decontamination Applications

Enzyme-based CBNR Countermeasures Under Development.

    a. Intravascular CWA Defense – Pre-exposure Prophylaxis
       (Nanotechnology Encapsulation – USAMRICD, Edgewood)
    b. Self Decontaminating CBW Reactive Paints and Coatings
       (Reactive Surfaces, Ltd. Austin, Texas)
    c. Non-caustic Medical Decontamination – Towelettes
       (LynnTech, Ltd., College Station, Texas)
    d. Non-caustic Physical Decontamination – Solution Formulations
       (Reactive Surfaces, Ltd., Austin, Texas)
    e. Aerosol Anti-CWA Fogging Technology
       (Pacific Northwest National Laboratory, DOE; Battelle, Inc.)
    f. Discriminating CWA Biosensors
       (Auburn University, PNNL, DOE)
    g. Primary Chemical Agent Demilitarization
       (RSL, EG&G – Toelle CMA)
    h. Secondary CMA Waste Products Decontamination
   (RSL, EG&G – Toelle CMA)

Task 8: Development of New Enzyme Biocatalysts for Detection and Destruction of Chemical Warfare

The pepQ prolidase from *E. coli* has been purified and been shown to catalyze the hydrolysis of dipeptides of the following structure (turnover numbers = approx. 200 s^{-1}.)
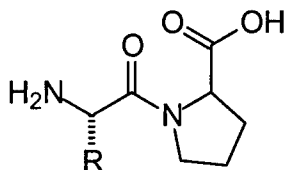
The purified enzyme will also catalyze the hydrolysis of organophosphate and organophosphonate esters. In every case the R_P-enantiomers are hydrolyzed faster than are the corresponding S_P-enantiomers. The protein has been crystallized and the seleno-methionine derivative has been prepared. We are confident that this enzyme will serve as another template for the enzymatic detoxification or organophosphate nerve agents. A model of this protein has been constructed using sequence identities with methionine amino peptidase and proline amino peptidase. The protein modeling has utilized the known three dimensional structures for these two proteins as

templates. We have also demonstrated for the first time that this enzyme will hydrolyze diisopropyl fluorophosphates as shown below:
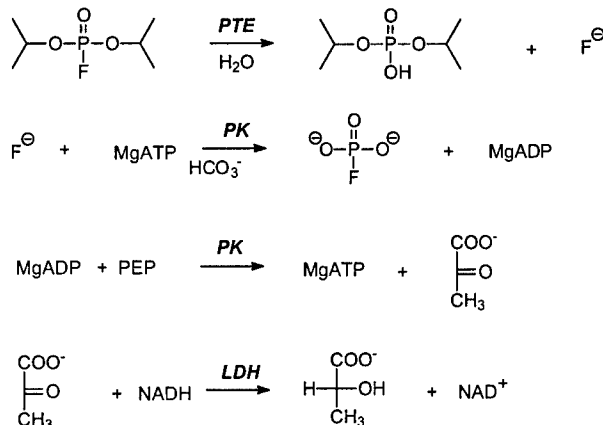
$$\text{(diisopropyl fluorophosphate)} \xrightarrow{\text{H}_2\text{O}} \text{(diisopropyl phosphate)} + F^{\ominus}$$
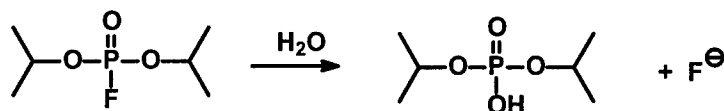
Hydrolysis of Diisopropyl Fluorophosphates

The wild type enzyme will also hydrolyze analogs of B, GD, GF, and VX.

Task 9: Development of a General Approach for High Throughput Screening of Mutant Enzymes for Remediation of Chemical and Biological Agents Using Arrays of Living Cells
We have developed a new high through put assay for the screening of intact cells and partially purified proteins that have the ability to hydrolyze organophosphates with phosphorus-fluorine bonds. In this assay we have taken advantage of the ability of the enzyme pyruvate kinase to phosphorylated fluoride anion in the presence of MgATP and bicarbonate. The products of this reaction are fluoro-phosphate and MgADP. The formation of MgADP is then coupled with pyruvate kinase and phospho(enol)pyruvate to form MgATP and pyruvate. Finally the pyruvate is coupled with lactate dehydrogenase in the presence of NADH to form lactate and NAD. The rate of the production of fluoride is monitored by following the conversion of NADH to NAD$^+$ at 340 nm. We are in the process of determining the optimal concentrations of bicarbonate, MgATP, PEP, and NADH so that there is a 1:1 conversion of fluoride to NAD. We are also determining the detection limits for fluoride for this assay. This new assay will likely be

Coupled Assay for Fluoride

$$\text{(organophosphate)} \xrightarrow[\text{H}_2\text{O}]{PTE} \text{(phosphate)} + F^{\ominus}$$

$$F^{\ominus} + MgATP \xrightarrow[\text{HCO}_3^-]{PK} {}^{\ominus}O\text{-P(=O)(F)-}O^{\ominus} + MgADP$$

$$MgADP + PEP \xrightarrow{PK} MgATP + \text{(pyruvate)}$$

$$\text{(pyruvate)} + NADH \xrightarrow{LDH} \text{(lactate)} + NAD^+$$

superior to the use of a fluoride electrode for monitoring the rate of formation of fluoride and likely be superior to our recently developed fluorescent assay for the formation of fluoride that can be used in a 96-well format for high throughput assays.

Task 11: Investigation of the Application of Polynuclear Metal Complexes for the Detection and Destruction of Chemical Warfare Agents (Gabbai)

Organopalladium complexes as catalysts for the hydrolysis of organophosphorus triesters

Thiophosphate derivatives such as parathion are pesticides which are typically not considered as chemical warfare agents. Nevertheless, they remain highly toxic to humans and could be used as chemical weapons by terrorist groups. Moreover, such pesticides remain widely available and their use for terrorist purposes has become a concern. For these reasons, we have devoted much of the past 12 months to the study of organopalladium complexes as catalysts for the hydrolysis of thiophosphate derivatives such as parathion. The formation of these palladium thiophosphate compounds reflects the thiophilic character of palladium and possibly accounts for the ability of such complexes to promote the hydrolysis of methylparathion. These findings suggest that thiophosphate complexes are possible intermediates in the catalyzed hydrolysis of methylparathion by cyclometallated palladium complexes.
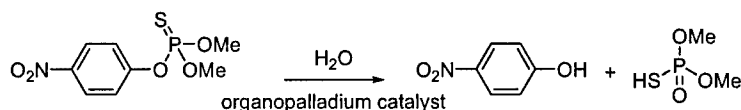
Hydrolysis of Dimethylparathion

In a continuation of these studies, we have screened the catalytic properties of various palladium complexes including the *ortho*-palladated complex (S,S)-di-□-(acetate)-bis{2-[2-(4-carbomethoxy)oxazolinyl]phenyl-C,N}dipalladium(II) (4) and [2-(2-pyridyl)-phenyl-C,N]palladium(II) thiophosphate (5). In the case of 4, catalytic hydrolysis only occurs at basic pH. At pH 9.0, the reaction rate has a first order dependence in substrate and palladium catalyst with a second order rate constant of 726 ($\pm$ 30) $M^{-1}s^{-1}$ which is remarkably high. In the case of 5, the reaction is catalytic under neutral or slightly acidic conditions which constitute a noteworthy improvement. At pH 7, the reaction rate has a first order dependence in parathion and palladium catalyst with a second order rate constant of 150 ($\pm$ 10) $M^{-1}s^{-1}$.
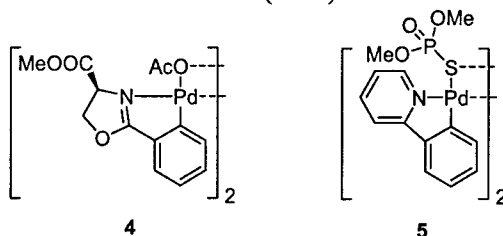

Structure of the monofunctional palladium catalysts investigated

No dinuclear complexes tried could parallel the activity of the monofunctional catalysts. *We have developed a catalyst (5) that effectively hydrolysis parathion at pH 7.*

Detection of chemical warfare agent surrogates:

We have developed a colorimetric assay for diisopropyl phosphorofluoridate (DFP, a sarin analog) in water at neutral pH. This assay is based on the synergy of 2-PAM, an organic hydrolysis catalyst, and 4-nitrophenyl *tert*-butyldimethylsilyl ether (9) which serves as a fluoride dosimeter. The interaction of DFP with catalytic amounts of 2-PAM results in rapid hydrolysis and production of fluoride. The latter reacts with 4-nitrophenyl *tert*-butyldimethylsilyl ether thereby triggering the release of the bright yellow *p*-nitrophenol.
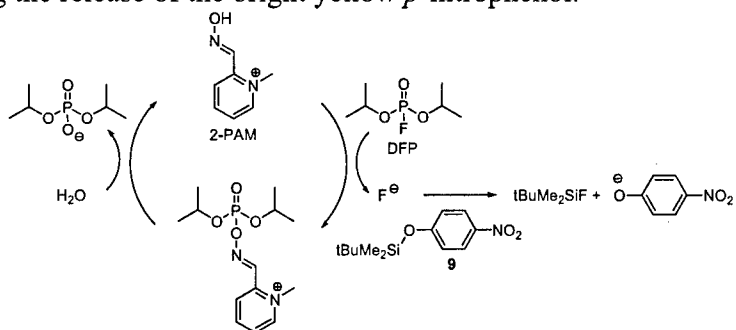
Task 13: Detection of Bacterial Pathogens Using Bacteriophage Arrays and Biofluorescence

There are seven genomovars of the *Burkholderia cepacia* soil bacterium, particularly of interest is the genomovar I plant pathogenic strain isolated from onion and soil samples, and the genomovar III, which is a related human pathogenic strain, causing infections in patients with cystic fibrosis. Several putative virulence factors, including two different exopolysaccharides (EPSI and EPSII /Cepacian/, have been purified from this bacterium. We have isolated three novel myophages, Bcep781 and Bcep43 that infect *B. cepacia* genomovar I and Bcep1 (genomovar III), producing a halo around the plaques. The halo formation indicates the presence of a depolymerase enzyme (dpo), capable of degrading the host EPS. Depolymerase activity was detected when Bcep781 ORF43 gene was expressed in E. coli from a pET-11a vector. Fusions with a Histidine-tagged sequence resulted in its purification on a cobalt affinity column. Thin layer chromatography was used to identify the hydrolysis products. Chemical mutagenesis will be used to create non-halo forming mutants, providing evidence for the association of the ORF 43 gene with the dpo activity. Electronmicroscopic (EM) analysis of the phage attachment to the bacterial exopolysaccharide has been initiated.

The enterobacterium *Erwinia amylovora*, the fire blight pathogen of rosaceous plants and pome fruit, produces copious amounts of extra cellular polysaccharide (amylovoran), which acts as a host specific toxin during pathogenesis. The *E. amylovora* bacteriophage ERA 103 produces clear plaques surrounded by halos when grown on the encapsulated host. Based on the sequence analysis of the ERA103 phage, the depolymerase gene was identified and cloned into the pET 11-a vector for expression and purification.

Erwinia amylovora bacteriophage ERA103 plaques surrounded by halos.

Task 15: Development of Integrated Microfluidic-based Sensors for Detection of Chemical and Biological Weapons

The overarching goal of our work is to develop new microfluidic biosensor systems for detecting chemical and biological threat agents. Such systems are envisioned to consist of three generic parts: sample preparation, analysis, and reporting. We have developed microfluidic devices for the simultaneous detection of multiple analytes based on multiple enzymes-entrapped hydrogel arrays. The fabrication of the hydrogel arrays relied on a microfludic network and photocrosslinkable hydrogel. The possible cross-talk between enzyme reactions occurring on the array type hydrogel patches was avoided by introducing PDMS microchannels between gel arrays. Different concentrations of glucose were sensed simultaneously using the microfludic device hosting glucose oxidase/horseradish peroxidase entrapped hydrogel arrays. The detection was made possible by observing fluorescence products of the two-step enzyme reactions. The simultaneous detection of glucose and galactose could be carried out by fabricating galactose oxidase/horseradhish peroxidase and glucose oxidase/horseradhish peroxidase enzymes entrapped hydrogel arrays. For another application, we demonstrated that organophosphate could be detected using three enzymes, i.e. acetylcholinesterase/choline oxidase/horseradish peroxidase entrapped in the gel arrays. This hydrogel array-based microfluidic device will provide more efficient way of detecting multiple analytes.

KEY RESEARCH ACCOMPLISHMENTS

The Texas A&M University System Digital EMS

- Development of additional PC104 based system modules
- Implementation of significantly smaller computer and communications packages.
- Design of personnel based digital still and video camera unit
- Design of new generation DTS – smaller, lighter, more function

The Texas A&M University System Detection and Remediation of Chemical and Biological Threat Agents Program

- Selected genetic constructions have been analyzed with actual CW agents and demonstrate significantly enhanced catalytic activity with Kcat values for VX that approximate 40 s-1 and for VR that exceed 200 s-1. The turnover values of these new enzymes are adequate for the development of enzyme-based destruction of VX and other V-type agents as well as the G-agents where they are 5-10 times faster.
- The formation of several different studies of genetic changes near and within the active site of OPH have been screened for improvements in the hydrolysis of a number of chiral analogs of the nerve agents; mutants that are an order magnitude faster than the wild type enzyme for the hydrolysis of VX analogs have been isolated.
- We have developed a new high through put assay for the screening of intact cells and partially purified proteins that have the ability to hydrolyze organophosphates with phosphorus-fluorine bonds. In this assay we have taken advantage of the ability of the enzyme pyruvate kinase to phosphorylated fluoride anion in the presence of MgATP and bicarbonate
- A sensitive, optical biosensor has been produced by forming a conjugate of the recognition enzyme OPH with fluorophore reporter elements CNF). This is the first successful demonstration of paraoxon detection by fiber optic system; the base is the commercially available Analyte 2000.
- New gene discovery with three different classes of OP neurotoxin degrading enzymes (OPAA, PON, and OPH) has produced the first novel enzymes in several years. The human serum detoxifying enzymes have been genetically cloned and sequenced: they are being over-expressed. OPAA families are being cloned and over-expressed.
- New microfluidic biosensor systems have been effective for detecting chemical and biological threat agents. Such systems consist of three generic parts: sample preparation, analysis, and reporting. We have developed micrfludic devices for the simultaneous detection of multiple analytes based on multiple enzymes-entrapped hydrogel arrays.
- A soft lithographic process based on microcontact printing (μCP) of organic monolayers, hyperbranched polymer grafting, and subsequent polymer functionalization, has resulted in polymer/n-alkanethiol patterns that direct the seeding of bacterial cells. These have been used for high through-put screening for new decontamination enzymes.

- Small molecules that should mimic the activity of OPH in the hydrolysis of organophosphorus nerve agents have been synthesized. The modified synthesis and design of chemical catalysts for the destruction of CW Agents has been effective and is being explored further as they have been shown to degrade CW surrogates.
- We have established a system for isolation, characterization and rapid DNA sequencing of phages for a model pathogen, Burkholderia cepacea. The next step is to identify appropriate phages for detecting B. cepacea using a phage-based lysis assay based on fluorimetry. Phage can be used to detect and inactivate biowarfare agents of categories A, B, and C.

REPORTABLE OUTCOMES

The Texas A&M University System Digital EMS

Presentations
- October 15 – 17, 2003 - Trinity Valley Exposition, Liberty, TX
- November 23 – 26, 2003 - Texas EMS Conference, San Antonio, TX
- December 18, 2003 - Demo for TAMU Director of Homeland Security
- February 19, 2004 - Demo TAMU Director of Homeland Security/Northrop Grumman
- March 23, 2004 – Demo to Impact Instrumentation/NASA Wyle Labs
- April 6, 2004 - State of Texas budget staff
- April 7, 2004 - Los Angeles Fire Chief
- April 8, 2004 – Smart Medical Technologies Summit 2004, Houston, TX
- May 2-4, 2004 – American Telemedicine Association, Tampa, FL
- June 21, 2004 - TX US Senator Cornyn staffer
- July 14, 2004 – Demo to Dr. Charles Galloway, Director DOD Chem/Bio
- August 10, 2004 – PLR in Frederick, MD
- August 15–18, 2004 - AFMESA Initial Concept Evaluation
- September 26–28, 2004 - Internet2 meeting in Austin, TX

Patents Filed
- Ewing, Richard E.; Duke, James H.; Wall, James A.; Flournoy, Larry D.; Kocmoud, Christopher J.; Xu, Zhihua; Salinas, Jose; Tindall, Robert D.; "Deployable Telemedicine System", Patent Application Filed March 18, 2004

- Ewing, Richard E.; Wall, James A.; Salinas, Jose; Goats, Lawrence S. III; Narayanan, Deepa; "Managing Data Packet Routing For Heterogeneous Communication Links", Patent Application Filed April 29, 2004

- Ewing, Richard E.; Duke, James H.; Wall, James A.; Flournoy, Larry D.; Kocmoud, Christopher J.; Xu, Zhihua; Salinas, Jose; Tindall, Robert D.; "Communicating Medical Information In A Communication Network", Patent Application Filed April 15, 2004

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Publications
1) Post-Genomic Chemistry", IUPAC Technical Report. International Union of Pure and Applied Chemistry. 2004, S.D. Varfolomeyev, E. Efremenko, I. Beletskaya, I. Bertini, G. M. Blackburn, A. Bogdanov, R. Cunin, J. Eichler, I. Galaev, V. Gladyshev, D. O'Hagan, T.

Haertle, J. Jarv, A. Karyakin, I. Kurochkin, M. Midolajczyk, V. Poroikov, I. Sakharov, F. Spener, N. Voyer, and J. Wild. In Press.

2) "Nanoparticle-based Optical Biosensors for the Direct Detection of Organophosphate Chemical Warfare Agents and Pesticides". 2004. A.L. Simonian, T. Good, S.-S. Wang, and J. R. Wild. In Press.

3) "A FET-based Biosensor for The Direct Detection of Organophosphate Neurotoxins. 2004. A.L. Simonian, A.W. Flounders and J. R. Wild. Electroanalysis. In Press.

4) "Electron beam irradiation as a protection against the environmental release of recombinant molecules for biomaterials applications." 2004. R. Shane Gold, Joseph Maxim, Douglas J. Halepaska, Jr. Melinda E. Wales, Douglas A. Johnson, and James R. Wild. 2005. Journal of Biomaterials Science, Polymer Edition 16:78-88.

5) J. Heo; G. H. Seong; W. Zhan; R. M. Crooks "A Microfluidic Biosensor Based on an Array of Hydrogel-entrapped Enzymes" (in preparation).

6) L. Sun; R. M. Crooks "Photonic Reporting of Electrochemical Reactions Using Light Emitting Diodes" *Anal. Chem.*, August, **2004** (submitted).

7) D. Liu; R. K. Perdue; L. Sun; R. M. Crooks "Immobilization of DNA onto Poly(dimethylsiloxane) Surfaces and Application to a Microelectrochemical Enzyme-Amplified DNA Hybridization Assay" *Langmuir* **2004**, *20*, 5905-5910.

8) W. Zhan; R. M. Crooks "Microfluidic Logic Circuits" *J. Am. Chem. Soc.* **2003**, *125*, 9934-9935.

9) M. Kim, Q. Liu, F. P. Gabbaï "Use of an organometallic palladium oxazoline catalyst for the hydrolysis of methylparathion" *Organometallics* **2004**, *23*, published online.

10) M. Kim, F. P. Gabbaï "Stoichiometric reactions of methylparathion with a palladium aryl oxime metallacycle" *Dalton Trans.* **2004**, 3403-3407.

Patents

- D. Albagli; L. Sun; R. M. Crooks "Photonic Signal Reporting of Electrochemical Events" CIP related to Patent 6 filed 22 July, 2003.

- W. Zhan; J. Alvarez; R. M. Crooks "Electrochemical Sensing in Microfluidic Systems using Electrogenerated Chemiluminescence as a Photonic Reporter of Electroactive Species" Filed 21 March, 2003. U. S. Patent Application Serial Number: 10/393,942.

CONCLUSIONS

The Texas A&M University System Digital EMS

Efforts throughout 2004 on the civilian DREAMS vehicle have been focused on continuing the development and testing of the field unit deployed in Liberty Co., TX. New communications environments were tested and fielded to adjust to specific requirements of rural areas.

The majority of all modules of the DREAMS software suite have been significantly enhanced in robustness and features this year as a result of continued research, development and Phase I testing. The user interface has been improved as a result of formal usability testing and new system features provide greater situational awareness and communication between the paramedic and remote physician. Integration of a 12-lead ECG and portable ventilator expanded the capability of DREAMS and support for a still image camera and Motorola VHF radios is coming in the near future.

General hardware developments have focused on reducing size, weight, and power requirements for the onboard ambulance systems. The initial mobile satellite system was completed and successfully tested. The terrestrial satellite terminal was completed and tested. Final modem upgrades will occur in FY2005. Applications have been made for extended FCC licenses for research.

A new design was begun on the DTS that significantly reduces size, weight, and power requirements. This design was specifically developed to provide convergent evolution with requirements in the civilian and military vehicles.

Work continued on the HMMWV DREAMS prototype. The design and system development was broadened to remove as much as possible any specific ties to the HMMWV itself. Parameters such as weight, power consumption, A/C needs, etc have been re-evaluated to be more generic and consistent with a "vehicle of opportunity" concept.

The Texas A&M University System Detection and Remediation of Chemical and Biological Threat Agents Program

As in previous efforts, the interdisciplinary research teams in the Detection and Remediation of Chemical and Biological Threat Agents Program of the DREAMS project have developed productive, widely-based interactions involving academic, federal, and private company scientists, engineers, and educators through the Texas A&M University System. Postdoctoral fellows and doctoral students are receiving invaluable training as they are involved in the

development of rapid detection systems for chemical and biological threat agents, contributing to and participating in first-responder emergency medical training, environmental detoxification, and equipment/personnel decontamination, as well as developing material for protecting America's food supply. What should be highlighted this year is the development of commercial collaborations and accomplishments.

1. Eclipse Sciences, Inc. was co-founded by Richard Crooks to commercialize technology developed under the DREAMS contract. Venture capital is currently being pursued in London, Cambridge, and Austin, Texas. Eclipse is developing a hand-held detector for detection of DNA and proteins associated with biological weapons of mass destruction.
2. Collaboration with Reactive Surfaces, LTD, of Austin, Texas, has resulted in the development of enzyme-based decontaminating slurries that are being evaluated by the Southwest Research Institute in San Antonio under contract with the CMA at Toelle, Utah. The enzyme is performing extremely well in Sarin and VX decontaminating trials. Similar studies have been obtained by a DARPA study with painted and solution decontamination at Dugway Proving Grounds.

The overall research has resulted in 10 peer-reviewed publications, numerous presentations, and multiple invited lectures in 2003-2004. They have presented two patents and leveraged their research activities into several million dollars in competitive research grants from the NIH, NSF, and several other federal funding agencies, such as DOE and DOD.